Virtual Polymerase Chain Reaction

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The polymerase chain reaction (PCR) stands among the keystone technologies of our time for analysis of biological sequence data. It is used in virtually every laboratory doing molecular, cellular, genetic, ecologic, forensic, or medical research. Despite its ubiquity, we lack the precise predictive capability that would enable detailed optimization of the dynamics of PCR reactions.

In this project, we are developing tools to perform virtual PCR (vPCR) by building new computational methods (Fig. 1) to model the kinetic, thermodynamic, and biological processes of PCR reactions. These tools will allow us to predict the effects of primers and reaction conditions on PCR products, and thus to optimize these variables. For the first time, the algorithms we are building will enable simulation of a complete thermocycle (ramp and soak) and product prediction for multiple thermocycles (Fig. 2), which no other software package to-date can do.

Project Goals

The result of this project, a suite of programs that predict PCR products as a function of reaction conditions and sequences, will be used to address outstanding questions in pathogen detection, forensics and microarray applications, as well as to enable detailed examination of the effects of contaminants and PCR inhibitors on the PCR process.

vPCR should enable scientists to optimize PCR protocols, in terms of time, temperature, ion concentration, and primer sequences and concentrations, and to estimate products and error rates in advance of
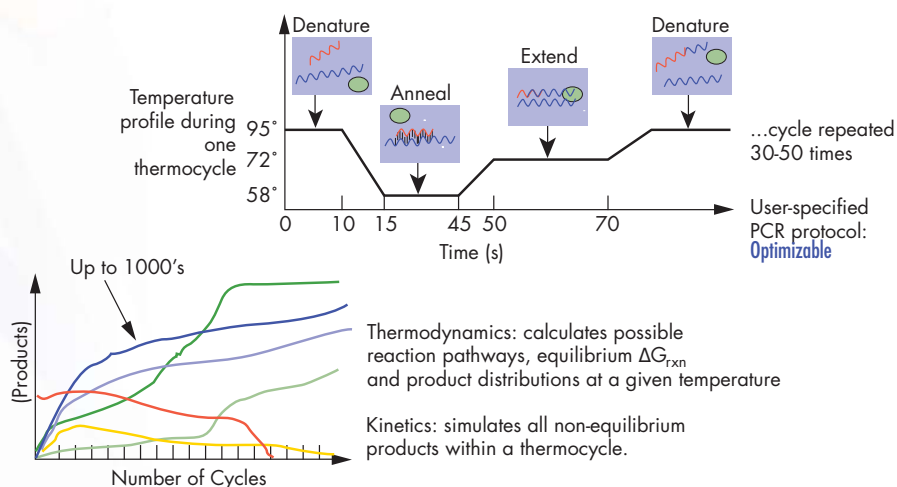

Figure 1. Flow chart showing how new capabilities will model important processes and predict the full spectrum of PCR products.

performing experiments. Our proposed capabilities are well ahead of all currently available technologies, which do not model non-equilibrium kinetics or polymerase extension, or predict PCR products. A provisional and a full patent application (IL-11192) have been filed.

Relevance to LLNL Mission

vPCR supports LLNL missions in homeland security, Genomes to Life (GtL), and human health. Any field that uses PCR, including bioforensics, biodetection, basic research in GtL, and disease research, such as cancer, will benefit. The challenges that vPCR can address include identification of genes and organisms present in complex microbial communities; optimization of DNA-polymerase-based gene synthesis; and forensic discrimination of closely related sequences.

FY2004 Accomplishments and Results

We have written the thermodynamics code to calculate reaction pathways and free energies, and have linked this with KINSOL, a nonlinear, simultaneous equation solver developed by CASC researchers, to solve the thermodynamic equations. We have developed the framework for efficient

data transfer between the kinetic and thermodynamic modules. For the kinetic simulations we have developed the algorithms and written prototype code to perform Gillespie's formulation of stochastic chemical kinetic simulations for the PCR process. Currently, reaction probabilities are based on annealing probabilities in terms of rates of nucleation, proximity effects, and free energies of reaction.

Related References

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FY2005 Proposed Work

In the next fiscal year we plan to add polymerization (extension) to the kinetic and thermodynamic simulations; modify the collective capability to handle denaturation of primers during a thermocycle (the reverse reaction); develop the capability to dynamically handle reaction vessel temperature ramps; optimize parallelization at multiple levels; perform experiments using real-time PCR to test the models; and prepare a manuscript for submission to a peer-reviewed journal.
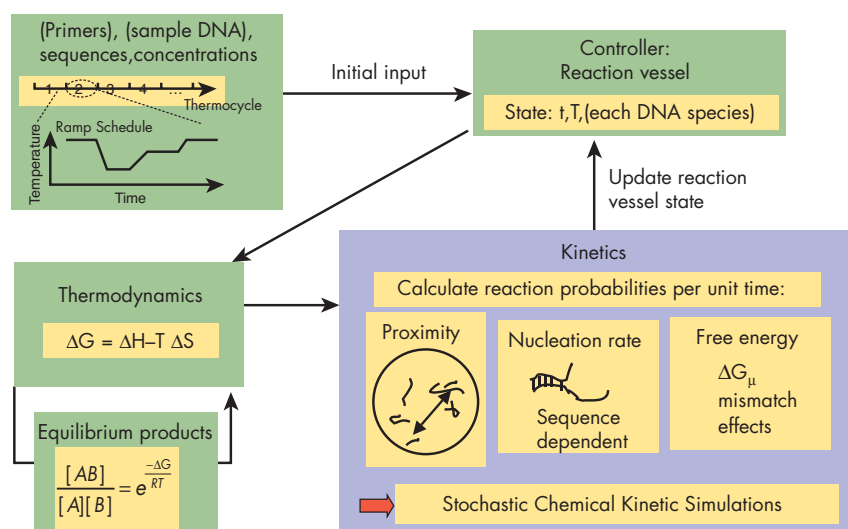
Figure 2. Flow chart showing how vPCR handles multiple cycles and adds kinetics to the simulation of the PCR process.